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ScienceDirect

Procedia Engineering 87 (2014) 292 – 295

Procedia Engineering

www.elsevier.com/locate/procedia

EUROSENSORS 2014, the XXVIII edition of the conference series

Bioconjugation of heavy metal-binding proteins on surface: an optical and gravimetric characterization

J. Politi[1,2*], A. Caliò[1,3], P. Dardano[1], M. Iodice[1], I. Rea[1] and L. De Stefano[1]

[1]*Institute for Microelectronics and Microsystems, National Research Council, Naples, Italy*
[2]*Department of Chemistry, University of Naples "Federico II", Naples, Italy*
[3]*Department of Physical Sciences, University of Naples "Federico II", Naples, Italy*

Abstract

A proper bioconjugation of molecular probes onto a transducer surface is a key point in developing new kind of biosensors. Porous Silicon (PSi) and quartz resonators (QR) are commonly used as transducers in biochemical sensors using spectroscopic reflectometry and quartz crystal microbalance (QCM). In this work, we studied bioconjugation of oligopeptides, namely Phytochelatins (PCs), which are able to bind heavy metal in aqueous solutions, both on PSi and QR surfaces: strong competition between protein-binding and protein-solubilization interactions are highlighted by both techniques.

Peer-review under responsibility of the scientific committee of Eurosensors 2014

Keywords: QCM, Porous Silicon, Bioconjugation, Biosensor, Phytochelatins.

1. Introduction

Biosensors are constituted by a bio-recognition element conjugated with a transducer element. A silicon-derived material as porous silicon (PSi) was widely studied in last twenty years due to its optical properties [1-4]. PSi is used as smart transducer material, since, on exposure to chemical substances, the average refractive index changes drastically [5]. Moreover, PSi exhibits a sponge-like morphology characterized by specific surface area up to 200 – 500 m^2 cm^{-3} [6] so that it can be very sensitive to the presence of biochemical species which penetrate inside the

* Jane Politi. Tel.: +390816132490; fax: +390816132598.
E-mail address: jane.politi@na.imm.cnr.it

Peer-review under responsibility of the scientific committee of Eurosensors 2014
doi:10.1016/j.proeng.2014.11.665

pores. PSi can be fabricated by electrochemical etching of doped crystalline silicon in hydrofluoridric acid (HF) [7]. Tuning etching process parameters (etch time, HF concentration, doping level, and so on) allows a modulating of PSi porosity which in turns permits the fabrication of multilayered optical structures as 64 layers Thue-Morse (T-M) filters. This optical structure, due to characteristic alternation of porosity layers, is more sensitive than symmetric multilayers such as microcavity or rugate filters [8], thus making easier the study of biomolecules immobilization. Furthermore, in recent years development of biosensors assigned an important role to Quartz Crystal Microbalance (QCM) technology. QCM is currently used in measuring with high sensitivity small mass changes, becoming a cost-effective tool. Standard nanogravimetry exploits the piezoelectric quartz resonators (QR) properties in quantifying the resonance frequency shift Δf when a mass m is adsorbed to or desorbed from their surface, according to Sauerbey's equation (1):

$$\frac{\Delta f}{f0} = \frac{\Delta \mathrm{m}}{\mathrm{A}\rho \mathrm{l}} \quad (1)$$

where $f0$ is the fundamental frequency of QR, A is the gold area, Δm is the adsorbed mass variation corresponding to frequency shift Δf and ρ and l are the quartz density and thickness, respectively [9-10].

The PSi and QR surfaces can be both chemically modified to obtain optical and nanogravimetric biosensors, respectively. Crucial point in biosensors development is the bioprobe choice that should be immobilized onto the transducer surface. One of the most useful biomolecular probes for heavy metal ions detection is a family of proteins which selectively binds them, named Phytochelatins (PCs). PCs are small, heavy metal-binding proteins with general structure of $(\gamma\text{-Glu-Cys})_n\text{Gly}$ (n=2-11) that complex with toxic metal ions protecting fungi and plants [11-13].

In this work we describe the bioconjugation of Phytochelatin 6 (PC_6) by a proper functionalization strategy on PSi T-M and QR surfaces for optical and nanogravimetric biosensors development.

2. Bioconjugation strategy

2.1. Porous silicon bioconjugation

Inorganic surfaces, such as porous silicon, need chemical modification for optical biosensors development due to highly hydrophobicity of as etched PSi devices that avoid infiltration of aqueous solution into sponge-like matrix [14]. The first passivation step is the oxidation of PSi in order to stabilize the surface. A previous study [15] was made to evaluate the spotting conditions of proteins bioconjugation on inorganic surfaces and to develop the chemical modification of PSi optical transducers represented in figure 1. The oxidized surfaces were treated by Piranha solution and aminopropyltriethoxysilane (APTES) to obtain a primary amine for homo-bifunctional cross-linker, in this case bis-sulfo-succinimydilsuberate (BS^3) linker. BS^3 brings a sulfo-*N*-hydroxysulfosuccinimide (sulfo-NHS) group that reacts with primary amines at pH 7-9 to form stable, covalent amide bonds, so it is able to bind primary amines on PSi surfaces, generated by silanization process, and primary amine of PC_6.

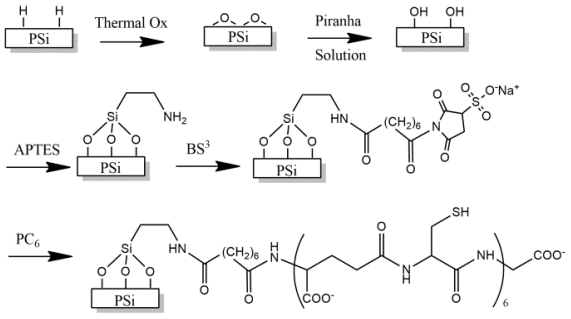

Fig. 1 Functionalization scheme of an as-etched PSi device from thermal oxidation to PC_6 bioconjugation

The functionalization process of porous silicon optical structure can be easily monitored by a label-free technique such as spectroscopic reflectometry. Data obtained by recording reflection spectra of T-M optical filters for each step of chemical modification are shown in Table 1.

Table 1. Spectroscopic reflectometry data recorded after every functionalization step reported for each sample

	T1 (1.5mg/ml)	*T2 (3mg/ml)*	*T3 (6mg/ml)*	*T4 (10mg/ml)*
Ox	943.6±0.1	942.3±0.5	948.2±0.2	950.4±0.4
APTES	977.0±0.3	974.4±0.4	982.3±0.2	985.3±0.1
BS3	992.8±0.4	991.4±0.3	996.9±0.5	1000.3±0.5
PC6	988.5±0.3	985.0±0.1	995.3±0.4	997.6±0.5

Data show that APTES red shift is of about 33nm for each device, while cross-linker red shift is of about 16nm, showing good repeatability of functionalization process. After bioconjugation of PC_6 a quite unexpected blue shift of the reflection spectra is revealed for each concentration of proteins.

2.2. Quartz resonators bioconjugation

QCM is an alternative way to characterize the same passivation process. QR surfaces were chemically modified by a very similar biomodification: we used the same conditions for cross-linker and proteins immobilization, but the golden area on QR was functionalized by a thiol-PEG-amine molecule (chemical structure showed in figure 2) that binds gold with thiol-terminal and BS^3 with amine terminal.

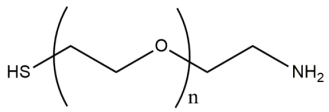

Fig. 2 Chemical structure of Thiol-PEG-amine

Each step of QR resonators passivation was monitored by QCM characterization. Figure 3 shows that the dynamic of functionalization until cross-linker reveals a frequency decrease which means a mass increase that demonstrated a successful binding of thiol-PEG-amine and BS^3.When PC_6 molecules interact with QR the data show an increasing of frequency, which means a mass decrease, according to blue shifts obtained by spectroscopic reflectometry data. In this case, the final oscillation frequency is still lower than the Thiol-PEG-amine-QR level so that we can conclude that the bioconjugation of PC_6 was at least partially obtained.

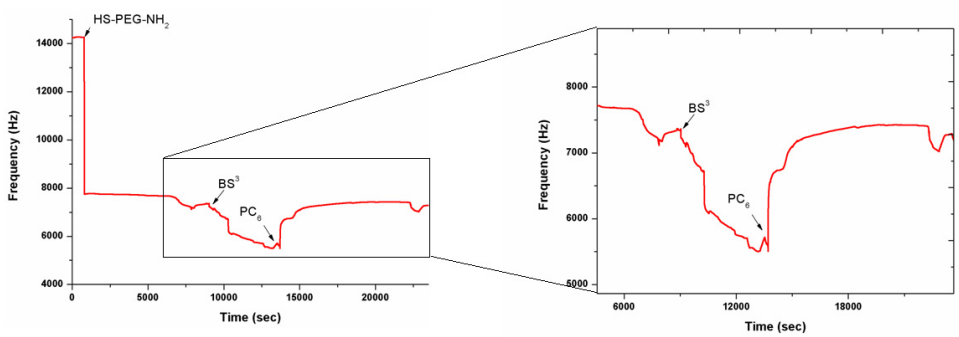

Fig. 3 Frequency variation for each chemical functionalization step made on QR (left) plus zoom of frequency variation after PC_6 bioconjugation

3. Conclusion

Biosensors development critically depends on effective bioconjugation of biomolecular probes onto trasducer surfaces. In this study, the conjugation of heavy metal-binding proteins such as oligopeptides, known as Phytochelatins, rich in cysteine able to sequester heavy metals (as lead, mercury and cadmium) has been monitored. Both optical and nanogravimetric characterization techniques reveal that PC_6 react with PSi and QR surfaces partially detaching the BS^3 crosslinker. QCM characterization highlights a different behaviour between silicon and gold surface immobilization. In the latter case, the bioconjugation of PC_6 was in part successfully obtained, while in the first case protein-solubilization interaction is much stronger than protein-linker binding.

Acknowledgements

The present work is supported by Italian National Operative Program PON01_01525 "MONitoraggio Innovativo per le Coste e l'Ambiente marino".

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